Claims

1. A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display on a viral particle, the zinc finger polypeptide comprising at least three zinc fingers, with one zinc finger having partially randomised allocation of amino acids being positioned between two or more zinc fingers having defined amino acid sequence, the partially randomised zinc finger having random allocation of amino acids at positions -1, +2, +3 and +6 and at least one of positions +1, +5 or +8, position +1 being the first amino acid in the α-helix of the zinc finger.

2. A library according to claim 1, wherein the partially randomised zinc finger has random allocation of amino acids at each of positions +1, +5 and +8.

3. A library according to claim ~~1 or 2~~, wherein the encoded partially randomised zinc finger comprises the zinc finger of the Zif 268 polypeptide.

4. A library according to ~~any one of claims 1, 2 or 3,~~ in a form suitable for cloning as a fusion with the minor coat protein of bacteriophage fd.

5. A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

screening against at least a portion of the target DNA sequence a plurality of zinc finger polypeptides having a partially randomised zinc finger positioned between two or more zinc fingers having defined amino acid sequence, the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the plurality of zinc finger polypeptides being encoded by a library in accordance with ~~any one of claims 1-4~~; and

selecting those nucleic acid sequences encoding randomised zinc fingers which bind to the target DNA sequence.

12 11 ~~58~~ 88

~~6~~. A method according to claim ~~5~~, wherein two or more rounds of screening are performed.

7. A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

comparing the binding to one or more DNA triplets of each of a plurality of zinc finger polypeptides having a partially randomized zinc finger positioned between two or more zinc fingers having defined amino acid sequence, the zinc finger polypeptides being encoded by a library in accordance with ~~any one of claims 1-4~~ claim 1, and

selecting those nucleic acid sequences encoding randomized zinc fingers exhibiting preferred binding characteristics.

8. A method according to claim 7, comprising a preceding screening step according to claim 5 or 6.

9. A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, the method comprising the steps of:-

screening nucleic acid sequences encoding randomized zinc fingers having desired bidning affinity by a method according to claim 5 or 6;

selecting certain of the screened randomized zinc fingers for analysis of preferred binding characteristics by the method of claim 7;

and combining those sequences encoding desired zinc fingers to form a sequence encoding a single zinc finger polypeptide having the desired binding specificity.

10. A method of designing a zinc finger polypeptide for binding to a particular DNA target sequence, wherein a plurality of sequences encoding individual zinc fingers selected by the method of claim 5 ~~and claim 7~~ are randomly combined in the appropriate order to

encode a plurality of zinc finger polypeptides, the zinc finger polypeptides being screened against the target sequence, that combination of zinc finger sequences encoding a zinc finger polypeptide having optimal binding characteristics being selected for use.

11. A DNA library consisting of 64 sequences, each sequence comprising a different one of the 64 possible permutations of a DNA triplet, the library being arranged in twelve sub-libraries, wherein for any one sub-library one base in the triplet is defined and the other two bases are randomised, the sequences being in a form suitable for use in the selection method of claim ~~7 or~~ 8.

12. A library according to claim 11, wherein the sequences are associated, or are capable of being associated, with separation means.

13. A library according to claim 12, wherein the separation means is selected from one of the following: microtitre plate; magnetic or non-magnetic beads or particles capable of sedimentation; and an affinity chromatography column.

14. A library according to ~~any one of claims 11, 12 or 13~~ wherein the sequences are biotinylated.

15. A kit for making a zinc finger polypeptide for binding to a nucleic acid sequence of interest, comprising: a library of DNA sequences encoding zinc finger of known binding characterstics in a form suitable for cloning into a vector; a vector molecule suitable for accepting one or more sequences from the library; and instructions for use.

16. A kit according to claim ~~15~~, wherein the vector ~~is capable of directing~~ the expression of the cloned sequences as a single zinc finger polypeptide.

17. A kit according to claim 15 ~~or 16~~, wherein the vector is capable of directing the expression of the cloned sequences as a single zinc finger polypeptide displayed on the surface of a viral particle.

18. A kit for making a zinc finger polypeptide for binding to a nucleic acid sequence of interest, comprising: a library of DNA sequences, each encoding a zinc finger ~~in a form suitable for screening according to the method of claim 5 or 6, and/or selecting according to the method of claim 7 or 8;~~ and instructions for use.

19. A kit according to claim 18, wherein the library of DNA sequences is in accordance with any one of claims 1 to 4.

20. A kit according to claim 18 or 19, further comprising a library according to any one of claims 11 to 14.

21. A kit according to ~~any one of claims 18, 19 or 20~~ further comprising appropriate buffer solutions and/or reagents for detection of bound zinc fingers.

22. A kit according to ~~any one of claims 18 to 21,~~ further comprising a vector suitable for accepting one or more sequences selected from the library of DNA sequences encoding zinc fingers.

23. A method of altering the expression of a gene of interest in a target cell, comprising: determining (if necessary) at least part of the DNA sequence of the structural region and/or a regulatory region of the gene of interest; designing a zinc finger polypeptide to bind to the DNA of determined sequence, and causing said zinc finger polypeptide to be present in the target cell.

24. A method according to claim 23, wherein the zinc finger polypeptide is designed in accordance with the method of any one of claims 5-10.

25. A method according to claim ~~23 or~~ 24, wherein the zinc finger polypeptide comprises one or more further functional domains.

26. A method according to ~~any one of claims 23, 24 or 25,~~ wherein the zinc finger polypeptide comprises a nuclear localisation signal so as to deliver the zinc finger

polypeptide to the ~~nucleus of the target cell.~~

27. A method according to ~~any one of claims 23 to 26~~ claim 24, wherein the zinc finger polypeptide comprises the nuclear localisation signal from the large T antigen of SV40.

28. A method according to ~~any one of claims 23 to 27~~ claim 24, wherein the zinc finger polypeptide is caused to be present in the target cell by delivery into the cell of DNA directing the intracellular expression of the polypeptide.

29. A method of inhibiting cell division by altering the expression of a gene in accordance with the method of ~~any one of claims 23 to 28~~ claim 27, wherein the gene is one involved in regulating cell division.

30. A method of treating cancer, comprising delivering to a patient, or causing to be present therein, a zinc finger polypeptide which inhibits the expression of a gene enabling the cancer cells to divide.

31. A method of modifying a nucleic acid sequence of interest present in a sample mixture by binding thereto a zinc finger polypeptide, comprising contacting the sample mixture with a zinc finger polypeptide having affinity for at least a portion of the sequence of interest, so as to allow the zinc finger polypeptide to bind specifically to the sequence of interest.

32. A method according to claim 31, wherein the zinc finger polypeptide is designed in accordance with the method of any one of claims 5 to 10.

33. A method according to claim ~~31 or~~ 32, further comprising the step of separating the zinc finger polypeptide (and nucleic acid sequences specifically bound thereto) from the rest of the sample.

~~Claim 32~~ claim 32

34. A method according to ~~any one of claims 31, 32 or 33~~, wherein the zinc finger polypeptide is bound to a solid phase support.

Amend 34

claim 14

17 ~~36~~. A method according to ~~any one of claims 31 to 34,~~ wherein the presence of the zinc finger polypeptide bound to the sequence of interest is detected by the addition of one or more detection reagents.

18 claim 14 ~~36~~. A method according to ~~any one of claims 31 to 35,~~ wherein the DNA sequence of interest is present in an acrylamide or agarose gel matrix, or is present on the surface of a membrane.

37. A zinc finger polypeptide capable of inhibiting the expression of a disease-associated gene, the zinc finger polypeptide being not naturally-occurring and is specifically designed, by the method of claim 5 ~~any one of claims 5-10~~, to inhibit the expression of the disease-associated gene.

38. A zinc finger polypeptide according to claim 37, capable of inhibiting the expression of an oncogene.

39. A zinc finger polypeptide according to claim 37 ~~or 38,~~ capable of inhibiting the expression of a BCR-ABL fusion oncogene.

40. A zinc finger polypeptide according to claim 37 ~~any one of claims 37, 38 or 39,~~ designed to bind to the DNA sequence GCAGAAGCC.

41. A zinc finger polypeptide according to claim 37 ~~or 38,~~ capable of inhibiting the expression of a ras oncogene.

42. A zinc finger polypeptide according to claim 41, designed to bind to the DNA sequence GACGGCGCC.

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